The Incidence and Burden of *Hymenolepis diminuta* Cysticercoids as a Function of the Age of the Intermediate Host, *Tribolium confusum*

Ronald J. Kelly[1], Dennis M. O'Brian, and Frank F. Katz
Seton Hall University, New Jersey

Abstract: The incidence and size of the larval tapeworm burden in young, middle-aged, and old confused flour beetles was studied. The influences of sex and length of starvation period were also observed.

Virgin beetles from young parents were permitted to feed for 24 hours on whole gravid proglottids and then returned to the medium for at least fourteen days prior to being preserved, dissected, and examined for cysticercoids. A quantitative approach to feeding eggs to the beetles was unsuccessful.

Old females generally had a significantly smaller burden and incidence of cysticercoids when compared with young or middle-aged females, whereas middle-aged males generally had a significantly higher incidence only when compared with young or old males.

Apparently an age resistance to the establishment of *H. diminuta* in *T. confusum* occurs in the females only.

INTRODUCTION

Since 1892, when Grassi and Rovelli first described the development of *Hymenolepis diminuta* in the insect intermediate host, there have been various investigations on hymenolepidids in insects. While there have been reports of vertebrate host age effects on adult hymenolepidid incidence and burden (Shorb, 1933; Hunninen, 1935), we have found no reports in the literature with regard to the age of the intermediate host on the larval stage of the parasite. Therefore, this study was undertaken with the intent of observing the incidence and size of the cysticercoid burdens in beetles of specific ages. Since host sex and length of starvation prior to exposure to the tapeworm's eggs may also influence the burden, these factors were also considered. The tapeworm *H. diminuta* and the beetle *Tribolium confusum* were selected since the former requires, as part of its life cycle, an arthropod intermediate host and the latter has been shown to serve well in this capacity.

MATERIALS AND METHODS

All stock cultures and experimental groups were raised under constant light at a temperature of 25°C. and a relative humidity of 71% in a medium consisting of 95% bleached Gold Medal Wondra® flour and 5% National Active Dry® yeast. The use of yeast-fortified flour had been proposed by Lund and Bushnell (1939). All stock and experimental beetles were put into fresh containers and medium every two weeks. Eggs randomly selected from a stock

[1] Present address: The Squibb Institute for Medical Research, New Brunswick, N.J.

culture were used to establish a population of beetles of known ages. Eight to eighteen days following oviposition of these beetles, eggs were collected and individually placed in 12 × 35 mm patent lip vials containing approximately 5 mm of medium. In this manner, selection of the first eggs laid by the beetles of known ages was avoided, since this selection may have detrimental effects on the offspring as shown in *Drosophila* (O'Brian, Yablonsky, and Gillooly, 1964).

The adult beetles that developed from these eggs were maintained as virgins in individual vials throughout the experiment. Three different age groups of adults were used: those that were young (four to five weeks), middle-aged (23 to 24 weeks), and old. Adult beetles were considered to be old at 47 to 51 weeks on the basis of parental age studies by Raychaudhuri and Butz (1965) and personal communication with Raychaudhuri (1964).

Each age group was further subdivided into those starved five to six days (Series I) and those starved seven to eight days (Series II). Following the starvation period, all beetles were allowed to ingest an undetermined number of eggs for a 24 hour interval by feeding on three or four freshly obtained whole gravid segments of *H. diminuta*. The male Sprague-Dawley rats from which the tapeworms were removed had been inoculated orally at five weeks of age with three to five cysticercoids dissected from infected meal beetles, *Tenebrio molitor* (Carolina Biological Supply Co.). The infections in the rats were five to fifteen weeks post-inoculation. Only those *Tribolium* which were observed to have fed on proglottids were used in the accumulation and analysis of the data. After exposure to the proglottids, the beetles were returned to vials containing fresh medium for a period of at least fourteen days prior to being preserved in 10% formalin. The preserved beetles were dissected and examined for cysticercoids.

The sex of the beetle was determined in the pupal stage by the method described by Park (1934), in the adult stage by the method described by Hinton (1942), and at the time of dissection.

RESULTS

Table 1 summarizes the incidence and size of the cysticercoid burdens in beetles which fed on gravid proglottids. The significance of the differences ($P < 0.05$) between any two average numbers of cysticercoids was determined by the "*t*" test (Youden, 1951, p. 25) and the trend between any two incidences of cysticercoids by the "Chi square" test (Hoel, 1960, pp. 157–163).

Since there was no difference in the incidence of cysticercoids between the populations starved five to six or seven to eight days, the data was pooled for analysis. Old females had a lower incidence when compared to young and middle-aged females, whereas in the male population the incidence was higher in the middle-aged group when compared to young and old beetles. With re-

Table 1. The burden and incidence of *Hymenolepis diminuta* cysticercoids in beetles of known ages. Mean values are given with the standard errors.

		No. of days starved					
		Series I, 5 to 6 days			Series II, 7 to 8 days		
Age	Sex	No. infected / No. that fed on proglottids	Percent infected	Average ± S.E.	No. infected / No. that fed on proglottids	Percent infected	Average ± S.E.
Young: 4 to 5 weeks	Females	26/33	78.8	7.0 ± 1.62	34/41	82.9	10.6 ± 1.72
	Males	28/39	71.8	6.5 ± 1.41	15/30	50.0	4.0 ± 1.14
Middle-aged: 23 to 24 weeks	Females	33/42	78.6	5.3 ± 0.81	35/42	83.3	7.0 ± 0.99
	Males	24/30	80.0	7.7 ± 1.36	29/33	87.9	7.5 ± 1.39
Old: 47 to 51 weeks	Females	11/28	39.3	0.8 ± 0.28	21/38	55.3	5.9 ± 1.83
	Males	34/64	53.2	5.0 ± 1.17	32/55	58.3	5.4 ± 1.57

spect to differences between the sexes, only old females had a lower incidence than middle-aged males. All these differences were found to be significant employing the difference in proportions using a binomial distribution (Dixon and Massey, 1957, pp. 232–233).

In Series I (five to six days starvation) the burden in old female beetles was significantly smaller than that of young and middle-aged females, and males of all ages. In Series II (seven to eight days starvation) the burden in old females was significantly smaller when compared to young female beetles only. Also, in this series, young males had a significantly lower burden when compared to young females and middle-aged males.

When comparing both series, only old females starved seven to eight days had a significantly greater burden than old females starved five to six days.

DISCUSSION

The lack of reports on the relationships of age and sex of intermediate hosts to their cysticercoid burdens makes this paper unique. It should be noted, preliminary results of this investigation have been reported in the form of an abstract (Kelly et al., 1966).

Age resistance to the establishment of *Hymenolepis nana* has been studied by Shorb (1933) in rats and mice and by Hunninen (1935) in mice only, and both found that older animals had a greater resistance to the tapeworm than younger animals.

In our study, middle-aged male *Tribolium* generally had a significantly higher incidence of cysticercoids than young or old males, whereas old females

generally had a significantly lower incidence and burden than middle-aged or young females. It seems, therefore, that an age resistance to the establishment of *H. diminuta* cysticercoids occurred in female *Tribolium*. Moreover, starvation affected the burden only in old females. This may indicate an increased susceptibility in old females to the tapeworm eggs as marginal food reserves are depleted.

Some work on the effects of age of the insect vector has been carried out with respect to protozoan parasites. Terzian et al. (1956) found that older *Aedes aegypti* mosquitoes were more resistant to *Plasmodium gallinaceum* than younger mosquitoes. However, physiological factors also played a role since particular diets resulted in aged mosquitoes being as susceptible to the parasites as young mosquitoes.

In the literature, one finds contradictions regarding the role of the sex of the intermediate host in its susceptibility to the parasites it transmits. Duke (1930, 1933) considered his work on tsetse flies showed females to be more susceptible than males to trypanosomes. However, according to Burtt (1946), Duke's (l.c.) data showed no significant differences.

With respect to helminths, it is of interest to note that a number of papers have been published on the relationships of nematode parasites to changes in sexual characteristics of insect hosts with some investigators reporting changes occurring more frequently in one sex than the other. In his extensive review of this subject, Wülker (1964, p. 589) notes, "It can be said, generally that, with regard to the gonads, the females are more changed, and with regard to the external sex characters, the males are more changed. However, exact investigations on the possible reasons for this difference and the opposite behavior of internal and external characters do not exist as yet."

In the investigation reported here, the beetles fed only on whole gravid segments, and therefore, the number of eggs ingested per beetle cannot be correlated with the cysticercoid burden. However, it should be pointed out that quantitative feedings were tried during the course of this experiment but were unsuccessful. Moreover, no reports have been observed in the literature where quantitative feedings of *H. diminuta* eggs to beetles have been employed. At the present time in this laboratory, successful feeding attempts have been obtained and will be reported at a later date (Levine, et al.)

It appears from our work and that of others that there is a need for more detailed studies on host-parasite relationships as presented and discussed in this report.

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